DATA EVALUATION RECORD
FRESHWATER FISH LC_{50} TEST
GUIDELINE 72-1

1. CHEMICAL: MTI

   Shaughnessey #: 107107

2. TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one; certificate reference number 0223.02. issue number 008); stated purity of 94.2%; buff colored powder; solubility of MTI in water at 20°C is quoted by Zeneca Specialties be >20% w/w

3. CITATION: G. C. Roberts, J. E. Caunter and J. M. Shearing. 1993. Acute Toxicity to bluegill sunfish (Lepomis macrochirus). EPA Guideline No. 72-1. Lab. Study No. BL4868/B; Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon TQ5 8BA, UK; Submitted by Zeneca Inc., Wilmington, DE 19897; MRID 43138711

4. REVIEWED BY:

   Joanne S. Edwards
   Entomologist
   Ecological Effects Branch
   Environmental Fate and
   Effects Division (7507C)

   Signature: Joanne S Edwards
   Date: 1/31/95

5. APPROVED BY:

   Leslie W. Touart
   Section Head
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   Signature: L W T
   Date: 2/21/95

6. CONCLUSIONS: This study is scientifically sound and satisfies the guideline requirement (Gdln 72-1) for a 96-hour static acute toxicity test with the bluegill. Based upon mean measured concentrations, the 96-hour LC_{50} of MTI is 2.1 mg a.i/l (1.6 - 3.2 C.I.). This classifies MTI as moderately toxic to the bluegill. The NOEC is 0.85 mg a.i/l.

7. ADEQUACY OF THE STUDY: Core

8. RATIONAL FOR CLASSIFICATION: N/A

9. BACKGROUND: New chemical submission.

10. MATERIALS AND METHODS:

A. Test Organisms:

Guideline Criteria	Reported Information
Species Preferred species is the bluegill (*Lepomis macrochirus*)	species tested was the bluegill (*Lepomis macrochirus*)
Mean Weight 0.5-5 g	Mean: 1.99 g Range: 1.05 - 3.46 g
Mean Standard Length Longest not > 2x shortest	Mean: 44 mm Range: 36 to 52 mm
Supplier	Sea Plantations Inc., Salem, MA
All fish from same source?	yes
All fish from the same year class?	yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	>14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	last medication given was diet containing 0.6 g of tetracycline in 100 g of Promin- this was fed to the fish 3X/day for five days over a 9 mo. period preceding test
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	24 hrs prior to test was food withheld; no feeding during test

Guideline Criteria	Reported Information
Pretest Mortality < 3% mortality 48 hours prior to testing	0% mortality prior to testing (2 wks prior to testing)

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	dechlorinated tap water supplied from a 100 m^3 reservoir; it was passed through activated carbon and dechlorinated using sodium thiosulphate
Does water support test animals without observable signs of stress?	yes
Water Temperature 17°C or 22°C	22°C ± 1°C (temperature controlled room)
pH Prefer 7.2 to 7.6	7.4 prior to test initiation
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	8.8 mg/l prior to test initiation
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	25.7 mg/L as $CaCO_3$ at beginning of test
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	glass vessels (68 l capacity)
Type of Dilution System Must provide reproducible supply of toxicant	static test

Guideline Criteria	Reported Information
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	not reported
Photoperiod 16 hours light, 8 hours dark	yes; 10 minute dawn/dusk transition periods
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	none used

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	-
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 0.3 mg ai/l
Control Mortality ≤ 10% if static, ≤ 5% if flow-through	0% mortality
Number of Test Organisms Minimum 10/level, may be divided among containers	10 fish per concentration level including negative (water) control
Test organisms randomly or impartially assigned to test vessels?	yes
Biological observations made every 24 hours?	yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h high, medium, and low doses of control	temperature, pH and DO content in each test vessel was measured at 24 hr intervals throughout the test; there was continual measurement of temperature in the dilution water control
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	yes (at 0,48, and 96 hrs)

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Control Mortality Not more than 10%	0%
Raw data included?	no
Signs of Toxicity (if any) were described?	yes
Physical/Chemical Measurements	DO ranged 5.4 - 8.8 mg/l pH ranged 6.7 - 7.4 temperature ranged 21.5 - 22.1 °C

B. Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	-	10	0	0	0	0

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
0.18	0.13	10	0	0	0	0
0.32	0.24	10	0	0	0	0
0.56	0.48	10	0	0	0	0
1.0	0.85	10	0	0	0	0
1.8	1.6	10	1	1	1	1
3.2	3.2	10	10	10	10	10

C. Statistical Results

Method: Moving Average Angle

96-hr LC_{50}: 2.0 mg ai/l 95% C.I.: 1.7 - 2.6 mg ai/L
NOEC: 0.85 mg ai/l

12. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE:

A GLP statement was included in the report indicating that the study was conducted according to the principles of GLP laid out by the UK Dept. of Health Compliance Programme (1989) (which is in accordance with the Organization for Economic Cooperation and Development (OECD) principles of GLP ISBN 9264 12367 9). A quality assurance statement was included in the report.

Based upon mean measured concentrations, the 96-hour LC_{50} was 2.0 mg a.i./l (95% C.I.: 1.7 - 2.6 mg ai/l). The NOEC was 0.85 mg a.i./l.

13. REVIEWER'S COMMENTS:

Verification of Statistical Results:

Parameter	Result
Binomial Test LC_{50} (C.I.)	2.1 mg ai/l (1.6- 3.2 mg ai/l)
Moving Average Angle LC_{50} (95% C.I.)	method not appropriate since less than 2 concentrations had % dead between 0 and 100

Probit LC_{50} (95% C.I.)	-
Probit Slope	-
NOEC	0.85 mg ai/l

Mean measured concentrations averaged 72, 75, 86, 85, 89, and 100% for nominal concentration levels 0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l, respectively. In the lowest concentration levels, the highest mean measured concentration obtained during the test divided by the lowest was greater than 1.5 (exceeded ASTM guidelines). This is not considered to be a study flaw, since no mortality occurred at the four lowest dose levels.

No other problems were noted with the study except for the following:

o it was indicated in the report that the last medication given to the fish was a medicated diet containing 0.6 g tetracycline.... It was not reported on what date this treatment ended.

o the biomass loading rate was not reported.

These deficiencies did not affect the overall quality of the study.

Adequacy of Study:

1. Classification: Core
2. Rationale: N/A
3. Reparability: N/A

14. Completion Date of One-Liner for Study:

jedwards MTI acute fish

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.2	10	10	100	9.765625E-02
1.6	10	1	10	1.074219
.85	10	0	0	9.765625E-02
.48	10	0	0	9.765625E-02
.24	10	0	0	9.765625E-02
.13	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 1.6 AND 3.2 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.103238

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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